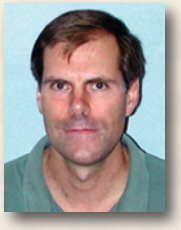
Hybridization, Regeneration, and Selective Release of DNA Microarrays



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DNA microarrays contain sequence-specific probes arrayed in distinct spots numbering from 10,000 to over 1,000,000, depending on the platform. This tremendous degree of multiplexing gives microarrays great potential for environmental background sampling, broad-spectrum clinical monitoring, and continuous biological threat detection. In practice, their use in these applications is not common due to limited information content, long processing times, and high cost. This work seeks to characterize the phenomena of microarray hybridization, regeneration, and selective release that will allow these limitations to be addressed. This will revolutionize the ways that microarrays can be used for LLNL's Global Security missions.

One study area is selective release. Microarrays easily generate hybridization patterns and signatures, but there still is an unmet need for methodologies enabling rapid and selective analysis of these patterns and signatures. Detailed analysis of individual spots by subsequent sequencing could potentially yield significant information for rapidly mutating and emerging (or deliberately engineered) pathogens. In the selective release work, optical energy deposition with coherent light is being explored to quickly provide the thermal energy to single spots to release hybridized DNA.

The second study area involves hybridization kinetics and mass-transfer effects. The standard hybridization protocol uses an overnight incubation to achieve the best possible signal for any sample type, as well as for convenience in manual processing. There is potential to significantly shorten this time based on better understanding and control of the rate-limiting processes and knowledge of the progress of the hybridization. In the hybridization work, a custom microarray flow cell will be used to manipulate the chemical and thermal environment of the array and image the changes over time during hybridization.

A related study area is regeneration. Microarrays cost hundreds of dollars and can typically be used only once, or at most a few times, due to degradation in signal with reuse. Balancing the thoroughness of the regeneration process against the stability of the microarray and quantitative signal requirements could enable new microarray applications.

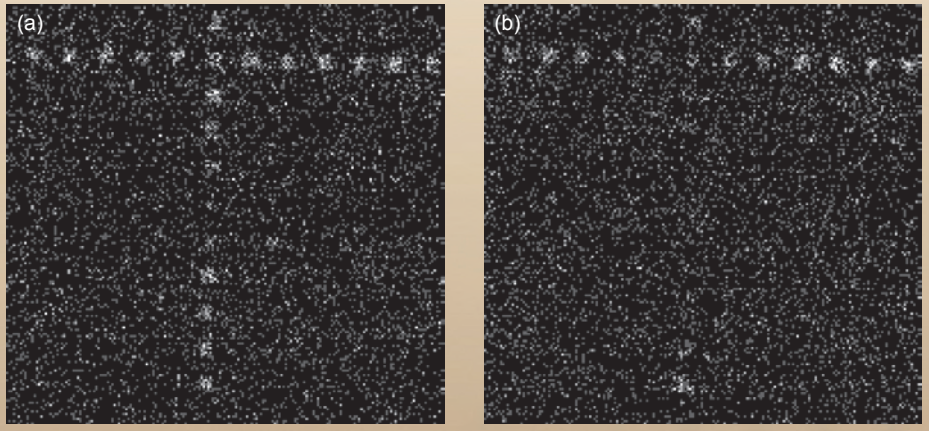

Figure 1. Demonstration of the effect of IR laser energy deposition on a DNA microarray at approximately 100-µm resolution from the spot field, (a) before and (b) after deposition.

Project Goals

The goals of the selective release work are to characterize the phenomena involved in high-resolution energy deposition with an IR laser and to demonstrate selective release of DNA from a microarray. This includes assessing the effects of wavelength, absorption, spot size, materials, pulse energy, and fluid flow.

The goal of the hybridization work is to quantify the rate-limiting processes in microarray hybridization and to

demonstrate improvement in hybridization time by controlling the process.

The goals of the regeneration work are to determine the cause of microarray degradation in regeneration and to reduce degradation.

Relevance to LLNL Mission

LLNL has ongoing efforts in detection methods against biological terrorism. The next stage of molecular diagnostics for biological threats is to look much more broadly for emerging threat bio-signatures, such as virulence elements or natural and engineered mutations. This capability is targeted against new natural pandemics and engineered biological warfare agents, while still detecting the full set of known bio-threat agents, to enable prompt countermeasures.

FY2009 Accomplishments and Results

Most of the effort this fiscal year was focused on selective release. We extended the capability of the DNA Release and Capture Laser (DRACULA) system with an alignment method and positioning controller to address microarray spots. With this system, we demonstrated energy deposition to microarrays by showing an effect on spot fluorescence near the diffraction limit, at approximately 100-µm resolution (Fig. 1). We developed a 3-D computational fluid dynamics (CFD) model to characterize the penetration heating profile of the current 10.6-µm CO_2 laser, and used the code to assess the energy deposition that would result from a new 1.47-µm diode laser (Fig. 2). This confirmed that the shorter-wavelength laser would provide better heating penetration,

Figure 2. Results from a numerical simulation of the temperature distribution resulting from (a) the 10.6-µm laser and (b) the 1.47-µm laser.

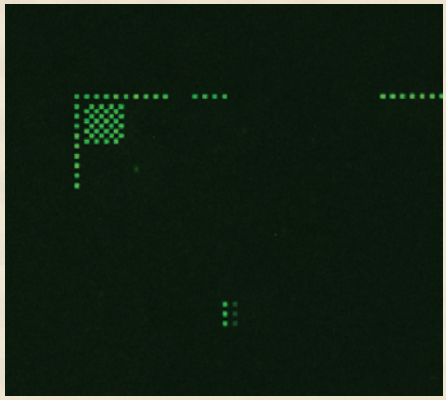

Figure 3. Detail image (approximately one two-hundredth of a microarray scan) showing 15-µm fiducial spots in an initial experiment of integrated hybridization and scanning using the microarray flow cell.

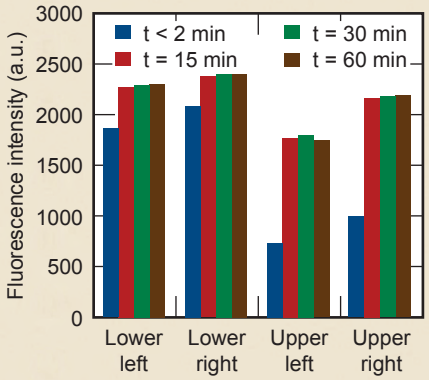

Figure 4. Average fluorescence values over time for the four fiducial corners during an initial experiment of integrated hybridization and scanning. Most of the signal was achieved early in the incubation.

which may be required for well-controlled selective release. We also designed and fabricated custom microarrays with SARS and Rhinovirus probes for use in selective release testing of biological samples.

In the first steps of the hybridization work, we used the microarray flow cell to perform hybridizations first off-scanner and then integrated with the scanner (Fig. 3). We developed an analysis method to quantify the hybridization level for this system, and proved that the experimental apparatus will be able to provide kinetic data by tracking hybridization level over time on control spots (Fig. 4).

Related References

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FY2010 Proposed Work

For selective release, the key milestone will be release and capture of undamaged SARS target DNA selectively over Rhinovirus target DNA. For the hybridization effort, we will design an array for hybridization rate tests investigating effects such as probe length, target length, and sequence mismatch, and will quantify those effects along with concentration, flow rate, and temperature. In regeneration, we plan to fabricate sets of arrays with custom coupling and probe chemistry to assess their stability to different regeneration processes in bulk and in the microarray flow cell. Combined, these efforts will expand the applicability of microarrays to LLNL's Global Security missions.